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Plant Symposia and Workshops

P-1

Evolution and Germplasm Conservation of Economic Groups of *Cannabis*. ERNEST SMALL. Agriculture and Agri-Food Canada, Saunders Building, Central Experimental Farm, Ottawa, ON, CANADA K1A 0C6. Email: ernie.small@agr.gc.ca

Humans have selected divergent kinds of cannabis plants (*Cannabis sativa*), differing in anatomy, chemistry and physiology, to supply diverse economic products. Currently, there is an explosion of societal, commercial and political support to develop the species commercially. Progress is hampered by widespread misunderstanding of the genetic relationships of the different classes of plant, and by unavailability of characterized germplasm collections which can be employed by breeders, agronomists and researchers in various disciplines for research and development. Plants selected for production of fiber from the stems were dominant for millennia, but the fiber market has limited prospects. Unfortunately most seed collections in public gene banks represent plants selected for fiber, and these have limited usefulness for the principal modern uses of cannabis: oilseed, medicinal cannabinoids, and recreational marijuana. All fiber plants are naturally tall, and so are "sativa type" marijuana strains, but from an architectural perspective this wastes energy by producing stem tissues that are of low value. Short-stature plants represent the most desirable architecture for modern usages. Germplasm for short stature is available from "indica type" and clandestinely bred marijuana strains, and far-northern low-THC plants. Since drug and oilseed plants are valued primarily for their female plants, elite biotypes may require maintenance and replication of vegetative tissues. Public gene banks are essential for the long-term development of *C. sativa*, and selection of germplasm meriting preservation requires knowledge of the evolutionary history of wild and domesticated kinds of the species.

P-2

Cannabis sativa L.: Botany, Chemistry and Drug Development. M.A. ELSOHLY. National Center for Natural Products Research and Department of Pharmaceutics and Drug Delivery, School of Pharmacy, University of Mississippi, University, MS 38677. Email: melsohly@olemiss.edu

Cannabis is one of the oldest medicinal plants known to man. The plant found its origin in Asia and its use spread to the Middle East and later to Europe and the USA. The plant has been recommended by many traditional healers and herbalists for the treatment of a variety of ailments such as headaches, asthma, diarrhea, constipation, pain, anxiety – to mention just a few. As a plant, Cannabis is a highly variable species. It belongs to family Cannabaceae. Whether the genus Cannabis contains one species or more has been a long matter of debate. Identifying the active ingredient(s) in Cannabis was not an easy task and the first work that came close to defining the skeleton of the active constituent(s) was in the 1940s but the actual structure of the active constituent (D^9-tetrahydocannabinol) was not determined till 1964. Scientific investigations with cannabis and the cannabinoids have exploded since then. A total of 565 constituents have been isolated from *Cannabis sativa* so far, out of which 120 are phytocannabinoids. This presentation will summarize Cannabis botany, species debate, potency, the progress of the work carried out with cannabis and the cannabinoids over the years and where this drug falls among all the medicines we have today to help in our fight against disease, and the rapidly changing landscape governing its control and use as a medicine.

P-3

Cannabinoid Dosage Formulations - From the Field, Pharmacy, Dispensary and Street. BRIAN F. THOMAS. Analytical Chemistry and Pharmaceutics, Room 187 William F. Little Medicinal Chemistry Building, 3040 E. Cornwallis Road, RTP, NC 27709–2194. Email: bft@rti.org

Cannabis sativa has a long history of use for a myriad of medicinal purposes, including use for its psychotomimetic effects. However, in the United States cannabis is classified as a schedule I controlled substance by the US Drug Enforcement Agency, indicating that it has no recognized medicinal value. Nevertheless, cannabinoid-derived pharmaceutical preparations are available in the United States and are regulated by the Food and Drug Administration (FDA)

and the Drug Enforcement Administration (DEA). In some states, patients and recreational users can also obtain cannabinoid-containing medications and recreational products in herbal form or in Cannabis-derived dosage formulations through dispensaries and statewide programs. Medicinal Cannabis or Cannabis-derived products available in these dispensaries have not been approved for use by the FDA and are still considered Schedule I controlled substances by the DEA. Due to a lack of registration, documentation, inspection, and approval of these products by the FDA, there is public concern about their quality, reliability, and safety. Indeed, these products have been distributed without safety packaging, tamper resistance, or clear unit doses and have been inadvertently consumed by both children and adults who have become intoxicated or suffered adverse effects. Even more alarming is the increasing prevalence and use of synthetic cannabinoids that are sprayed on herbal products and subsequently smoked for their marijuana-like intoxicating properties. Originally developed for the legitimate research purpose of furthering the understanding of the cannabinoid system, these synthetic cannabinoids are being abused worldwide, creating issues for regulatory and law enforcement agencies that are struggling to keep up with the growing number of compounds of various structural motifs.

P-4

The Role of Local Auxin Biosynthesis in Plant Development. ANNA N. STEPANOVA. Department of Plant and Microbial Biology, Program in Genetics, North Carolina State University, Raleigh, NC 27695–7614. Email: atstepan@ncsu.edu

The plant hormone auxin is a key regulator of a plant's life, from seedling germination to fruit ripening. The prevalent form of auxin, indole-3-acetic acid (IAA), is synthesized from the amino acid tryptophan via a two-step pathway, catalyzed by aminotransferases TAA1/TARs and flavin monooxygenases YUCs. The discovery of the intricate spatiotemporal expression patterns of the *TAA1/TAR* and *YUC* auxin biosynthetic genes refuted earlier views that IAA is made in shoot meristems and is then distributed to other parts of the plant via polar auxin transport. More recent data implicated local auxin biosynthesis [that acts in concert with auxin transport] in the establishment and maintenance of morphogenic gradients essential for plant development. We are employing a variety of experimental techniques and systems, from ectopic expression to grafting, from whole plants to excised organs and cell populations, to delineate the role of local auxin biosynthesis and its regulation in plant growth and development in *Arabidopsis*.

P-5

Regulation of Anthocyanin Biosynthesis in the WD40-bHLH-MYB Complex-Programmed *Arabidopsis* Cells. DE-YU XIE. Department of Plant and Microbial Biology, North Carolina State University, Raleigh, NC 27695. Email: dxie@ncsu.edu

To date, biosynthesis of anthocyanins is intensively characterized in model and crop plants. In particular, each biosynthetic step has been genetically and biochemically elucidated, which have greatly enhanced numerous successes of metabolic engineering. It is globally interesting that the biosynthesis of anthocyanins is limited to certain types or groups of plant cells. Although a few types of transcription factors (such as MYB, bHLH, and WD40) have been characterized to form complexes to regulate the activity of the biosynthetic pathway, whether one complex is sufficient for positive regulation remains open for studies. To understand this unknown, we developed red cell culture from an *Arabidopsis thaliana pap1-D* mutant, which is characterized by a high production of anthocyanins. Genome-wide transcriptomic analysis and other transcriptional characterizations have revealed that the anthocyanin biosynthesis in red cells is only controlled by the expression of *PAP1 (MYB75)*, *TT8*/*GL3 (bHLH)*, and *TTG1* (*WD40*), indicating that these transcription factors form the only WBM complex and one regulatory complex is sufficient to activate the biosynthesis of anthocyanins in plant cells. Based on this red cell culture model, we have further determined that the regulation of anthocyanin biosynthesis by different abiotic factors is dependent upon the expression of the WBM complex in red cells.

P-6

A New Balancing Act: Melatonin and Serotonin as Mediators of Plant Morphogenesis. LAUREN A. E. ERLAND and Praveen K. Saxena. Gosling Research Institute for Plant Preservation (GRIPP), Department of Plant Agriculture, University of Guelph, Guelph, ON, CANADA. Email: lerland@uoguelph.ca

Melatonin (Mel) and serotonin (5HT) are indoleamines first identified as neurotransmitters in vertebrates; they have now been found to be ubiquitously present across all forms of life. Though Mel and 5HT possess important roles in plant growth and development, their roles in morphogenesis are still poorly defined. We hypothesize that Mel and 5HT function as a novel class of plant growth regulators (PGRs). To investigate this, we used a dual approach: phytochemical analysis and in vitro culture

experiments. First, a simple and efficient method for the phytochemical analysis of Mel, 5HT and several established classes of PGRs, was developed for in vitro grown plants. Second, we examined the morphogenetic effects of Mel and 5HT in several plant culture systems including breadfruit (*Artocarpus altilis*) and St. John's wort (*Hypericum perforatum*: SJW). In breadfruit, though Mel had a minimal effect on growth, 5HT appeared to act as both an anti-browning agent and to possess cytokinin-like effects in culture. Particularly, it was found that 5HT (100 μM) could replace kinetin supplementation, in multiplication medium. Our lab possesses unique lines of SJW, a model for the study of Mel and 5HT, with high (L4) and low (L112) endogenous Mel levels, in comparison to wild-type plants. Neither root, nor shoot explants of the three lines showed a significant difference in growth. Grown on media supplemented with Mel, 5HT or their precursors (5, 10 or 30 μM), both root and shoot cultures showed a dose-dependent morphogenetic response, particularly with respect to shoot and root initiation. High levels (10–30 μM) of these compounds induced a generally inhibitory effect, while low concentrations (5–10 μM) showed improved growth and regeneration. Additionally, L4 showed inhibition at lower levels than did L112, supporting the dose dependent nature of Mel and 5HT, a defining characteristic of PGRs. Together this research presents a) a platform for the investigation of Mel and 5HT in morphogenesis, and b) suggests Mel and 5HT should be classified as a novel class of PGRs.

P-7

Role of Antioxidants in In Vitro Plant Culture Systems. PRAVEEN K. SAXENA. University of Guelph, Guelph, ON, CANADA. Email: psaxena@uoguelph.ca

The process of initiation and establishment of plant tissue cultures consists of the isolation of explants and optimization of culture conditions permitting the induction and expression of morphogenetic competence. In many species, these steps expose plant cells, tissues, and organs to extreme stresses caused by explanting injury, chemical toxicity, and abnormal culture environments. The reactive oxygen species generated in response to multiple stresses have been recognized as one of the most detrimental factors leading to recalcitrance of in vitro plant culture systems. Antioxidants have been shown to neutralize the damaging effects of reactive oxygen species and alleviate culture induced oxidative stress. This presentation will provide an overview of recent research on the role of antioxidants, particularly indoleamines such as melatonin and serotonin, in regeneration and conservation of a diverse range of plant species.

P-8

In Vitro Ploidy Manipulation for Ornamental and Bioenergy Crop Improvement. D. H. TOUCHELL and T. G. Ranney. North Carolina State University, Department of Horticultural Science, Mountain Horticultural Crops Research and Extension Center, 455 Research Drive, Mills River, NC 28759–3423. Email: darren_touchell@ncsu.edu

In vitro regeneration systems provide a powerful tool for manipulating ploidy level to assist with the breeding and development of new ornamental and bioenergy crops. The development of polyploids may increase ornamental characteristics and environmental tolerances, expand breeding opportunities, assist with the development of non-invasive triploid cultivars, and restore fertility in sterile hybrids. In vitro chromosome doubling is commonly induced using antimitotic agents such as cochicine, oryzalin and triflurilin. Successful induction is dependent the length of exposure and concentrations of antimitotic agents, explant types and interactions with basal media and plant growth regulators. Current research is focused on optimizing and exploring the effects of in vitro polyploidy induction on taxa with ornamental and bioenergy applications including *Miscanthus*, *Saccharum*, *Acer*, *Hydrangea*, *Rudbeckia*, *Rhodendron*, *Liriope*, *Ophiopogon, Zenobia* and *Magnolia*. In vitro conditions vary between taxa and individual genera, species and cultivars often require unique treatments to optimize polyploidy induction. In some taxa, the induction of polyploidy influences in vitro growth, development and root formation, leading to further studies on regeneration and rooting. Here we provide an overview of the application of in vitro chromosome doubling for crop breeding and improvement.

P-9

High Throughput Transformation Systems. J. ERIC GULLEDGE, Cassandra Collins, Jessica Johnson, Marina Kalyaeva, and Les Pearson. ArborGen, Inc., 2011 Broadbank Ct., Ridgeville, SC 29472. Email: jegulle@arborgen.com

With the explosion of available genome and gene information there exists an overabundance of gene candidates of predicted, but unverified, or unknown function that are of interest to researchers. Such candidate genes may prove to be important in understanding key metabolic processes or be the foundation for enhancing or developing new and desirable traits. A key tool in understanding candidate gene function is the ability to produce translines with genes inserted in different configurations: overexpression; under expression/knockout; ectopic expression; or even potential modifications to DNA/protein sequences with the aid of gene editing methods. However, with

such an overwhelming supply of candidate genes, testing such large numbers necessitates the application of efficient and cost-effective transformation systems. For evaluating genes from woody plants it is clearly most desirable that such transformation systems be based on woody species where the functionality of the gene can be assessed in an appropriate physiological background. With this in mind we have developed an efficient high throughput transformation system for a model tree species, *Populus deltoides*, and have used this system to develop translines representing over 600 gene constructs as part of a multi-institutional research effort, the BioEnergy Science Center (BESC), focused on understanding and eliminating biomass recalcitrance to improve biofuel yields. In addition we describe advances with transformation systems for other commercially important forest tree species.

P-10

Biotech and Genome Editing Regulations. ALAN McHUGHEN. University of California, Riverside, CA. Email: alanmchughen@ucr.edu

Several nations are reviewing regulations governing the environmental (cultivation) and commercial release of crops developed using gene editing or "New Breeding Technologies". Scientific societies focus on the technical aspects of product safety and make policy recommendations from that perspective. However, non-scientist groups often consider non-technical factors and make recommendations based on political, economic or social priorities. The tension can result in differing regulatory policies in different jurisdictions. These differences have led to problems in international trade with 'traditional' genetically engineered (transgenic) commodity crops. And incompatible regulations promise the same international trade disruptions with crops developed using (non-transgenic) gene editing techniques. This presentation will review the current regulatory status of NBT in several important trading partner countries and the prospects for international trade harmony.

P-11

Engineering the DNA of Our Governance Systems: Biosafety Best Practices and Issues for GMOs. T. KUIKEN. North Carolina State University, Genetic Engineering & Society Center, 1070 Partners Way, Raleigh, NC 27695–7565. Email: tkuiken@ncsu.edu

Over the last year, nearly one billion dollars were invested in synthetic biology companies (Synbiobeta, 2017). More and more start-up companies are entering the market providing designer organisms, which are changing the way traditional chemicals, materials, crops and medicines are being developed, produced and regulated. Meanwhile, genomic applications designed to combat human/animal disease and agricultural pests are challenging regulatory structures that were designed over 30 years ago. Society is rapidly moving from applications (and in some instances companies) that traditionally were treated as point source pollution towards living, moving targets. In addition, lower barriers to entry and access to funding (through crowdfunding platforms) has expanded who can participate in genetic engineering, further challenging the regulatory system. Over the last few years, U.S. federal agencies have been reviewing their authorities under the Coordinated Framework for the Regulation of Biotechnology. The European Commission released three opinions on synthetic biology (definition, risk assessment methodologies and research priorities), and the United Nations Convention on Biological Diversity expanded its Ad-Hoc Technical Expert Group on Synthetic Biology to examine gene drives and the impact of digital DNA. How the U.S. and other international governing bodies coordinate and address new genomic technologies will impact how they are developed, who is able to maneuver the system and thus participate in the market, and whether applications are introduced at all. New governance models will be needed to establish appropriate norms for government funding and regulatory agencies, for researchers within and outside traditional laboratory settings, and for crowdfunding platforms. This talk will explore how the U.S. and international governance systems are trying to keep pace with emerging genetic technologies, the biosafety/biosecurity issues related to them and how it could influence the field moving forward.

P-12

Regulatory Considerations for Genome Editing in Crops. MIGUEL E. VEGA-SANCHEZ[1] and Raymond Dobert[2]. Monsanto Company, [1]Global Breeding Technology Solutions, Genomics and Reproductive Biology Platform, 700 Chesterfield Parkway W, Chesterfield, MO 63017 and [2]Global Regulatory Policy and Scientific Affairs, 800 N. Lindbergh Blvd., Creve Coeur, MO 63141. Email: miguel.e.vega-sanchez@monsanto.com

Recent scientific advancements have led to the development of methods, collectively knows as New Breeding Techniques (NBTs), that offer a significant improvement over traditional breeding tools in terms of precision, efficiency, and speed. In particular, targeted mutagenesis via genome editing processes (CRISPR/Cas9, TALENs) can significantly improve the ability to generate new sources of genomic variation and useful traits. One of the characteristics of certain applications of

genome editing is that the resulting plant product does not contain transgenes, potentially avoiding the costly and lengthy process of obtaining global regulatory approvals associated with genetically engineered (GE) crops. Many processes for delivery of the gene editing machinery have been developed, such as the intermediate use of transgenes encoding the nucleases, or the direct use of ribonucleoproteins, all leading to null segregants (i.e. plants containing an edit without recombinant DNA insertion). As such, genome-edited plants can be indistinguishable from crops generated using conventional breeding or random mutagenesis techniques that are exempt from GE regulation in most countries. However, at this time, there is no global regulatory consensus on the status of gene-edited crop products, posing the potential for inconsistent regulatory approaches around the world which could negatively impact the deployment of these techniques. We will present an update on the global regulatory status of gene-edited products, with an emphasis on the implications of these decisions on the application of science-based policy and the future of these technologies.

P-13

Bentgrass Goes AWOL: A Story of Gene Flow, Research, and Regulation. C. AUER. University of Connecticut, Department of Plant Science, 1390 Storrs Road, U-4163, Storrs, CT 06269. Email: Carol.Auer@uconn.edu

The escape of glyphosate resistant creeping bentgrass (GRCB) from seed production fields in Oregon has led to years of stakeholder conflict and unsuccessful mitigation efforts. In part, this situation is the result of insufficient knowledge about the bentgrasses (*Agrostis*) and their biocontainment. Bentgrasses are a large and adaptable group of native and non-native grasses, and they have many characteristics that promote gene flow and weediness in North America. A proposal for GRCB commercialization in 2002 led to ecological risk assessment studies on species distribution, habitat suitability models, glyphosate selection pressure, and gene flow. These projects were conducted in suburban landscapes and golf courses where GRCB would likely be marketed and planted in the Northeastern US. The first study was a survey showing distribution of 8 bentgrass species and high co-occurrence between weedy creeping bentgrass (CB) and its relatives. A study around a surburban golf course revealed 4 non-native and 2 native bentgrasses in various plant communities. A habitat suitability model for the study site showed that 36% of the area provided suitable bentgrass habitat, and this habitat overlapped with areas occupied by threatened or endangered species. A retrospective gene flow study compared SSR markers in CB weed populations with CB cultivars on a golf course. Analysis showed that 3.3% of the CB weeds were related to golf course cultivars. A field experiment with CB and redtop showed that glyphosate treatment improved survivorship, growth, and reproductive potential compared to plants in unsprayed plots. Taken together, these research projects strongly suggested that the release of GRCB would allow two events: an exposure pathway through gene flow to feral CB and its relatives, and hazards from altered plant communities that support threatened and endangered species. Today, herbicide resistant bentgrasses continue to grow without control and GRCB (event ASR368) has been deregulated by USDA. Government documents suggest that GRCB seed will never be sold. However, this decision has drawn criticism because it implies unquantified future costs for seed producers, land managers, and the environment.

P-14

RNAi and Gene Editing as Tools for Containment of Genetically Engineered and Exotic Forest Trees. STEVEN H. STRAUSS, Amy L. Klocko, Estefania Elorriaga, Cathleen Ma, Michael Nagle, Haiwei Lu, and Anna Magnuson. Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR 97331. Email: Steve.Strauss@OregonState.Edu

Forest trees often have feral or wild relatives, can spread pollen and sometimes seed over long distances, and as keystone species can have large ecological impacts. Even when planted and highly managed, they are also viewed as part of the natural environment by many, and thus the spread and introgression of genetically engineered and exotic forms can be contentious. To mitigate these concerns, we are seeking to produce complete and stable sexual sterility for vegetatively propagated forest species, with a focus on poplar (*Populus*) and eucalypts (*Eucalyptus*). We will focus on our work field testing strategies for RNA interference against floral regulatory genes and overexpression of regulatory genes that prevent onset of reproduction. We will also review recent work with CRISPR-Cas gene editing to mutate genes essential for male and female reproduction. Results suggest that RNAi and overexpression can be highly effective, with little to no negative vegetative impacts and high trait stability among vegetative propagules, and over years, in the field. CRISPR-Cas studies have shown very high rates of small and large indels that are expected to stably and predictably impair fertility.

P-15

Evaluating Unintended Open Reading Frames in Genetically Modified Plants. H. HART. Syngenta, 9 Davis Drive, Durham, NC 27709. Email: hope.hart@syngenta.com

Molecular characterization is one part of the science-based, multi-disciplinary approach used in food, feed, and environmental safety assessment of genetically modified crops. A thorough understanding of the impact of the transgenic DNA insertion into the plant's genome helps to identify potential unintended effects of the transformation on the host plant and if they exist, to determine their impact on the risk assessments of the GM crop product. Part of the molecular characterization data package is the identification of new open reading frames (ORFs) and assessment of their potential to result in unintended proteins. The presence of unintended ORFs in the inserted DNA is not a safety risk in itself since their potential translation products are only hypothetical. The data requirements to identify and characterize ORFs are not harmonized among global regulatory bodies. Criteria used to identify, evaluate the likelihood of expression, and assess the impact, if any, of new ORFs on safety assessments are presented here.

P-16

Regulatory and Risk Assessment Issues Associated with Environmental Releases of Genetically Engineered Plants. JOHN M. CORDTS. Cordts Consulting LLC. Email: jmcordts@aol.com

Plant scientists are familiar with the numerous mechanisms available to plants that facilitate gene flow among and between sexually compatible species. One of the most readily identifiable means is that of pollen movement, intentional as used by plant breeders, and unintentional as occurs naturally by wind, water, and numerous types of pollinators. With the development of genetic engineering (GE) techniques, which ushered in the ability to move genes among any species desired, many governments have chosen to regulate these technologies and/or their products and control how and when developers can utilize GE plants in the environment. As such, not only are developers required to confine or contain their regulated GE plants, but they are also required to prevent or minimize exposure of such plants to non-GE sexually compatible species to minimize the likelihood of producing hybrid GE/non-GE plants that can persist in the environment. Pollination biology and plant harvesting and handling procedures are generally evaluated by regulators to determine the appropriate methods to confine regulated field trials. Plant biologists and regulators recognize that achieving absolute containment of GE plants in open air field trials is nearly impossible and at the same time regulators are tasked with allowing field trials without undue regulatory burden. Regulators, lawmakers, scientists, developers, and the public, therefore, are tasked with finding the proper balance between appropriate regulatory oversight that still allows and encourages innovation and scientific advancement. The discussion will also include the differences in regulatory oversight between non-regulated and regulated GE plants in today's global marketplace.

P-17

Cannabis In Vitro Propagation-A New Crop in an Experienced Industry. W. GRAHAM. Pure Food Gardening/Microclone Propagation, Belmont, CA. Email: bill@planttc.com

Since the cultivation of medical and recreational cannabis became legal in Canada, Isreal and parts of the United States, producers have used traditional propagation methods such as cut and stick cloning and some seed propagation. However, due to the need of higher production of plants required, the need for disease free stock, and the preservation of genotypes, the higher demands are being explored through the use of tissue culture propagation and maintenance. Government policies across the globe have historically restricted nearly all cultivation of this medically and scientifically valuable crop. Therefore, the practice of cannabis tissue culture has been neglected as so many of the world's other valuable crops have been introduced and studied in vitro for decades. As such the body of published research on cannabis tissue culture from academia is very limited and there is only a hand full of commecial tissue culture labs that practice and investigate cannabis propagation. The refinements and improvements developed by these institutions remain mostly unshared. An overview of best practices aquired from industrial labs for the micropropagation of Cannabis sativia will be presented.

P-18

Optimizing Operation Processes Through Quality Management Systems. THOMAS SHIPLEY. Canopy Growth Corp, Smiths Falls, ON, CANADA. Email: tom@canopygrowthcorp.com

Developing operational processes in a strict regulatory environment while scaling at a rapid pace can challenging. This presentation covers the way Quality Management Systems can be utilized not only for regulatory compliance, but to optimize operational practices and facilitate business scaling - reducing the cost of goods sold and allowing the business to become the leader in a newly emerging market.

P-19

Names, Strains and Claims, Oh My! Incorporating the Use of Genetic Analysis in a Budding Industry. ANNA SCHWABE and Mitchell E. McGlaughlin. University of Northern

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Presently in the United States, *Cannabis sativa* is federally illegal and listed as a Schedule 1 substance. However, due to recent state legislation changes, 8 states and Washington DC allow legal recreational *Cannabis* consumption, and 28 states allow *Cannabis* use for treatment of medical symptoms. The change in legal status of *Cannabis* has resulted in an unprecedented surge of new named strains, which are commonly described as Sativa, Indica, or Hybrid, based on morphology and reported effects. Given that strains are propagated from clones or seed from self-fertilization, strains should be essentially identical, no matter the source. However, consumers report that strains do not always elicit the same effects, especially when obtained from different sources, which begs the question, are samples of *Cannabis* strains obtained from various sources genetically comparable? In this study, genetic data from ten nuclear microsatellites was used to examine the genetic relatedness of 122 samples from 30 strains obtained from 20 recreational and medical dispensaries in Colorado, California, and Washington. As other studies have shown, the multiple genetic analyses performed here found no genetic distinction between described Sativa, and Indica type, or Hybrids thereof. Additionally, of 12 popular strains from multiple dispensaries, only 1 strain showed clear genetic consistency, 7 strains had at least one obvious genetic outlier, while 4 strains showed no genetic consistency among samples. Variations within strains showed no patterns and differences are found among states, among cities within the same state, among dispensaries in the same city, and surprisingly even between samples of the same strain from the same dispensary. These results demonstrate that strains are inconsistent and that the public view of Sativa/Indica type is not genetically supported.

P-20

The Molecular Mechanism Behind Hydrophobic Barrier Formation to Confer Salt Tolerance in Plants. Pannaga Krishnamurthy, Ho Wan Jing, Felicia Lok Chien Joo, Felicia Lee, Chiang-Shiong Loh, and PRAKASH P. KUMAR. National University of Singapore, Department of Biological Sciences, 10 Science Drive 4, Singapore 117543 SINGAPORE. Email: dbskumar@nus.edu.sg

Salinity is an abiotic stress that affects the growth and productivity of plants worldwide. Mangrove trees such as *Avicennia officinalis* exhibit remarkable ability to grow in saline environment by means of various adaptations. The roots of *A.officinalis* can exclude ~95% salt with the help of enhanced hydrophobic root barriers (Casparian bands and suberin lamellae). Cytochrome P450s play a key role in biosynthesis of suberin precursors. We identified several cytochrome P450 (CYP) genes that were differentially expressed upon salt treatment in the roots of *A.officinalis*. We used an *Arabidopsis* mutant, *atcyp86b1* to characterize the function of *CYP86B1* in regulating suberin biosynthesis. The *atcyp86b1* mutant seedlings showed salt sensitivity with reduction in root elongation. When treated with salt, their roots exhibited reduced suberin lamellae and Casparian bands. Heterologous expression of the coding sequence of *A.officinalis CYP86B1* in *atcyp86b1* resulted in rescuing of the salt sensitive phenotypes, indicating the involvement of CYP86B1 enzyme in suberin biosynthesis. In an effort to understand the underlying molecular regulatory mechanism of suberin biosynthesis, we identified specific WRKY transcription factors as the upstream regulators of *CYP* genes. These findings reveal how hydrophobic barrier formation is controlled to confer salt tolerance in plants. *Funding acknowledgement: This research grant is supported by the Singapore National Research Foundation under its Environment and Water Research Programme and administered by the Environment & Water Industry Programme Office (EWI) of the PUB, Singapore, NRF-EWI-IRIS (R-706-000-010-272 and R-706-000-040-279).*

P-21

Small Molecules, Big Impacts - MicroRNAs in Perennial Grass Development and Stress Response. H. LUO, S. Yuan, M. Zhou, N. Yuan, D. Li, Z. Li, and Q. Hu. Department of Genetics and Biochemistry, Clemson University, Clemson, SC 29634. Email: hluo@clemson.edu

MicroRNAs (miRNAs) are important regulatory noncoding RNAs involved in plant development, and plant response to environmental stresses. However, their specific roles in perennial grasses and the underlying mechanisms remain largely unknown. We have studied a number of conserved miRNA genes in creeping bentgrass (*Agrostis stolonifera*) and demonstrated that they are all regulated by environmental stimuli. Overexpression of these miRNAs causes pleiotropic phenotypes, including changes in root and leaf development, tillering, biomass production, and transition from vegetative to reproductive growth and vernalization response. Up-regulation of these genes also leads to significantly enhanced plant tolerance to salt, drought, heat and nitrogen deficiency, associated with changes in diverse physiological parameters. Gene expression analysis indicated that specific miRNA target genes were impacted by miRNA overexpression and some of the targets are directly involved in plant response to

abiotic stresses and nutrition deficiency. Manipulation of individual miRNAs also affected expression of other stress-responsive genes and miRNAs suggesting miRNA-mediated coordination of multiple stress regulators in plant stress response. Collectively, our results establish key roles of miRNAs in plant development and stress response of perennial grasses and suggest novel molecular strategies for use in crop genetic engineering for enhanced abiotic stress tolerance.

P-23

MS Media Kit and Design of Experiments (DOE) Overview. RANDALL P. NIEDZ. USDA-ARS, US Horticultural Research Laboratory, Ft. Pierce, FL. Email: randall.niedz@ars.usda.gov

A vigorous and functional in vitro system matches the environmental conditions and media components to the requirements of the plant species. Because in vitro systems are complex, determining the environmental conditions and inorganic and organic media components to achieve healthy growth can be time-consuming and difficult. Determining the nutritional requirements of a plant species is a multivariate problem best studied using a multivariate approach such as DOE. Mineral nutrition is fundamental to all plant tissue culture systems, but the mineral nutrient component alone includes 16 essential nutrients. Phytotechnology Laboratory's Deconstructed MS Media Kit represents a DOE approach to in vitro mineral nutrition research. The MS Media Kit includes premade salt stocks to formulate the multiple treatments combinations of a DOE experiment. The presentation will include 1) the geometric basis of DOE; 2) a geometric framework for mineral nutrient research; 3) the MS Media Kit and its relationship to mineral nutrient geometry and in vitro multivariate research; and 4) the use of the MS Media Kit to characterize and identify improved mineral nutrient formulations of micropropagated citrus.

P-24

The *Phyto*Technology Laboratories Deconstructed MS Media Kit. ANDREW J. DILLON, David S. Hart, and Kenneth C. Torres. *Phyto*Technology Laboratories, 14610 W. 106th, Lenexa, KS 66215. Email: Andrew.Dillon@phytotechlab.com

*Phyto*Technology Laboratories has recently released a kit called Deconstructed MS for the optimization of nutrient levels in plant tissue culture media. This kit contains six different solutions: a 100x ammonium nitrate solution (Group I); a 50x potassium nitrate solution (Group II); a 10x 'Mesos' solution containing calcium chloride, potassium phosphate, and magnesium sulfate (Group III); a 100x micronutrient solution without iron (Group IV); a 10x iron-EDDHA solution (Group V), and a 100x iron (II) sulfate/EDTA chelate solution (Group V). This kit can be used to recreate the ion concentrations typically found in Murashige & Skoog, and can be utilized to create a customized media for optimal growth of your species of interest. As a demonstration, a media optimization experiment was designed using Design of Experiment (DOE) principles, to produce a medium for strawberry (*Fragaria x ananassa*), and to provide an example of the applications of the Deconstructed MS kit.

P-25

Case Study #2: Using the Deconstructed MS Medium Kit to Evaluate Factors Influencing In Vitro Growth in Endangered Exceptional Species. VALERIE C. PENCE[1], Linda Finke[1], and Randall P. Niedz[2]. [1]Center for Conservation and Research of Endangered Wildlife (CREW), Cincinnati Zoo & Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220 and [2]USDA-ARS-U.S. Horticultural Research Laboratory, 2001 South Rock Road, Ft. Pierce, FL 34945. Email: valerie. pence@cincinnatizoo.org

One of the challenges of ex situ conservation of endangered exceptional plants is the heavy reliance on in vitro methods. While in vitro propagation protocols have been developed for many plants, some species still remain either totally or partially recalcitrant to culture. CREW's In Vitro Collection, which includes a wide variety of endangered species, provides a unique opportunity to study the in vitro growth of species from extreme environments. The Deconstructed MS Medium Kit was used with a 5-factor 2-level fraction design to study the growth of several species from the Collection, all from drier habitats, applying the same experimental set-up to each. The 5 factors tested included NH_4NO_3, KNO_3, mesos, minor elements, and Fe. The 16 media tested all included MS organics, 0. 5 mg/L BAP, 3% sucrose, and 0. 25% gel, all in unvented culture tubes. Shoot cultures of *Cycladenia humilis* var. *jonesii*, which can display an extreme form of hyperhydricity, showed few differences on any of the media, with all shoots remaining hyperhydric and showing similar relative growth. Shoot-forming callus of *Arctomecon humilis* showed more overall growth with more organized growth at lower NH_4NO_3 and Fe levels. Shoots of *Hedeoma todsenii* showed the most differences, both in growth and in the development of hyperhydricity. Higher concentrations of mesos and Fe had the greatest effects on increasing growth in general and in the height of the shoots. NH_4NO_3 was a major driver of increasing hyperhydricity both in stem and leaf tissue, with smaller effects from other factors. These results will be discussed in terms of identifying both the drivers of normal growth as well as the relationship of different morphological features associated with

growth in these species and will be compared with other DOE experiments in this laboratory. As the DMM Kit facilitates the application of the DOE approach to more species, accumulating data on the effects of media components on in vitro growth should lead to more efficient and effective ex situ conservation of endangered exceptional species.

P-26

Screening Experiments for Mineral Nutrition Using Deconstructed MS: Observing Subsequent Effects on Rooting and Acclimatization. JEFFREY ADELBERG, Department of Plant and Environmental Sciences, Clemson University, Clemson, SC 29634. jadlbrg@clemson.edu

Mineral nutrition in plant tissue culture is provided by a combination of salts; the Murashige and Skoog (MS; 1962) salt formulation is the most common. Many other salt-based formulations have been developed using responses measured in the laboratory. Mineral nutrition is also critical when the microcuttings are transferred from the laboratory to the greenhouse for rooting and acclimatization. A poorly developed root system has little ability to take up nutrients. Nutrients in the plant tissue are critical for subsequent development of roots, cuticle and photosynthetic competence during acclimatization and these largely come from the laboratory medium. None of the prior nutrient salt media were formulated for this purpose. The deconstructed MS media kit will be used to set up a 5-factor, response surface experiment for promoting rooting and acclimatization upon subsequent transfer to peat-based soilless mix. The mineral composition of the stage III medium will be evaluated based on survival, rooting, leaf and stem growth during the 5 week period subsequent to transfer. Our design requires 28 treatments – 23 being unique, including MS run in triplicate, and 3 true replicates. This design will assess the relative importance of all 5 nutrient groups, interactions among them, estimate optimal values of nutrient concentrations if they fall within the ranges tested, compare successful treatments to the control (MS), and assess random error within a treatment. Error control in the greenhouse environment is critical for this type of approach to work, and methods to this problem will also be discussed.

P-27

Technological and Health Relevant Attributes of Spray Dried Blueberry Polyphenol-protein Aggregates. ROBERTA T. P. CORREIA[1,2], Mary H. Grace[2], Debora Esposito[3], and Mary Ann Lila[2]. [1]Laboratory of Food Bioactive Compounds, Chemical Engineering Department, Federal University of Rio Grande do Norte (UFRN), Campus Central, 59078, BRAZIL; [2]Plants for Human Health Institute, Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, 600 Laureate Way, Kannapolis, NC 28081; and [3]Plants for Human Health Institute, Department of Animal Science, NC State University, 120 Broughton Drive, Raleigh, NC 27695. Email: rtcorrei@ncsu.edu

Phytoactive components from fruits and pomace can be rapidly degraded with exposure to light, heat and oxygen. Anthocyanins, in particular, are known for their susceptibility to pH change, among other factors (Patras, Brunton, & O'Donell, 2010). Therefore, processing techniques that stabilize these compounds and extend their shelf life while maintaining their bioactive properties would broaden opportunities for fruit processors, and potentially expand markets for fruit-based products. In this regard, an efficient strategy using protein-rich flours to sorb and complex polyphenols from fruit extracts was developed to create stable dry granular functional ingredients which provide a highly bioavailable delivery system for bioactive compounds (Roopchand *et al.*, 2012; Ribnicky *et al.*, 2015; Schneider, Esposito, Lila, & Foegeding, 2016). In this study, we demonstrate that spray drying, one of the most popular industrial-scale drying methods, is a convenient time-effective strategy to produce blueberry polyphenol-protein aggregates with desirable technological and shelf life attributes. The technological aspects are shown and discussed, as well as the anti-inflammatory activity, glucose metabolism and wound healing properties of these phytochemical-rich products.

P-28

Dietary Long-chain PUFAs Enrich Porcine Alveolar Macrophages and Modulate Inflammatory Response. KATHLEEN R. WALTER[1,2], Lin Xi[1], Sheila K. Jacobi[3], Debora Esposito[1,2], and Jack Odle[1]. [1]Department of Animal Science, North Carolina State University, Raleigh, NC 27695–7621; [2]Plants for Human Health Institute, North Carolina State University, Kannapolis, NC 2808; and [3]Ohio State University, Department of Animal Science, Wooster, OH 44691. Email: krwalte2@ncsu.edu

The US swine industry experiences severe production and profit losses from respiratory infections despite the wide spread use of vaccines and antibiotics. Long-chain PUFAs can modulate immune cell function through the production of eicosanoids, such as prostaglandin E_2 (PGE_2) which is capable of stimulating both inflammation and inflammatory resolution upon infection. The aim of this study was to determine the effects of dietary arachidonic acid (ARA) and eicosapentaenoic acid (EPA) on cultured porcine alveolar macrophages (AM) stimulated with LPS. Day-old pigs (n=60) were fed milk replacer for

21d. Diets contained 0.5% ARA (control), 2.5% ARA or 3.5% EPA of total fatty acids. Average body weight and clinical hematology were unaffected by diet treatments. Concentration of ARA (*w*/w) in AM from pigs fed 2.5% ARA had an 82% increase in ARA compared to controls, and the concentration was decreased 21% in pigs fed 3.5% EPA compared to control ($P < 0.01$). Lung tissue showed a similar enrichment pattern; lung tissue was enriched by 39% in pigs fed 2.5% ARA, while pigs fed the 3.5% EPA diet had a 45% decrease in ARA compared to control ($P < 0.002$). Media concentration of PGE_2 was increased following LPS stimulation of cultured AM. The increase in PGE_2 concentration was greater in macrophages enriched in ARA, exceeding that from control cells by 187% and that from EPA-enriched cells by 191% ($P < 0.05$). An increase in mRNA expression was observed with Cox 2, ALOX 12/15, and TNF-alpha upon LPS stimulation; while a decrease in ALOX 5 expression was observed. There were no significant changes in the generation of ROS or oxidative stress ($P > 0.05$). These data demonstrate dietary supplementation of LC-PUFAs can effectively alter lung tissue and alveolar macrophage fatty acid composition. Furthermore, these data validate that increased dietary ARA is an effective means to alter eicosanoid production and subsequently modulate inflammatory response in porcine alveolar macrophages up LPS stimulation.

P-29

Berry Extracts to Protect Skin from Inflammation. SIERRA BONNEY[1,3], D. Esposito[1,3], M. Grace[1,2], S. Komarnytsky[1,2], and M. A. Lila[1,2]. [1]Plants for Human Health Institute, NC State University, 600 Laureate Way, Kannapolis, NC 28081; [2]Department of Food, Bioprocessing, and Nutrition Sciences, NC State University, 400 Dan Allen Drive, Raleigh, NC 27695; and [3]Department of Animal Science, NC State University, 120 Broughton Drive, Raleigh, NC 27695. Email: sabonney@ncsu.edu

Among many traditional applications, berry preparations have been used for the treatment of various skin conditions. These mixtures are known to exhibit a variety of anti-inflammatory and anti-microbial activities, which are the desired properties for wound healing interventions. Given the increasing interest in the identification of biologically active plant constituents for wound healing and cosmetic applications, the objective of this study was to employ activity-guided screening techniques to 3 Alaskan berry extracts: *Empetrum nigrum* (crowberry), *Vaccinium uliginosum* (bog berry), and *Vaccinium vitis-idaea* (low-bush cranberry). The results of the skin cell migration assay, modeling the promotion of wound healing, revealed bog blueberry as the most potent berry sample. Complex flavonoid structures including proanthocyanin oligomers (PACs) were highly abundant in wild berry germplasm (tundra berries from the Alaskan coastal areas) and demonstrated some of the most potent efficacy in this model. Next, the underlying mechanisms by which PACs or their constituents produce a beneficial effect in fibroblast and keratinocyte cell cultures were examined. This data suggested that berry extracts accelerate wound healing in part by increasing cell proliferation and bioenergetics in fibroblasts, having the highest impact at the early phases of wound closure. Targeting cell bioenergetics with PACs may represent a promising method to alleviate delayed wound healing and improve cosmetic outcomes of wounds. This research approach is a prelude to establishing potential cosmeceutical applications using native Alaskan berries as a prime ingredient.

P-30

Bitter Receptors Control Glucose Absorption in the Gut by Modifying the GPCR Signaling Cascade. KIMBERLY M. PALATINI JACKSON[1,2], Thirumurugan Rathinasabapathy[1,3], Sierra Bonney[1,4], Debora Esposito[1,4], and Slavko Komarnytsky[1,2]. [1]North Carolina State University, Plants for Human Health Institute, 600 Laureate Way, Kannapolis, NC 28081; [2]North Carolina State University, Department of Food, Bioprocessing, and Nutrition Sciences, 400 Dan Allen Drive, Raleigh, NC 27695; [3] International Medical University, Department of Pharmaceutical Chemistry, School of Pharmacy, 126, Jalan Jalil Perkasa 19, Bukit Jalil, 57000 Kuala Lumpur, MALAYSIA; and [4]North Carolina State University, Department of Animal Science, 120 Broughton Drive, Raleigh, NC 27695. Email: palatini-jackson@ncsu.edu

Taste perception is an important aspect of food intake and may also influence carbohydrate absorption during digestion due to the localization of taste receptors throughout the gastrointestinal tract. Whereas bitterness has long been assumed an evolutionary signal for toxicity, current research suggests activation of bitter Type 2 Receptors (T2Rs) influence glucose absorption and utilization in human and animal models. No mechanism has yet explained these effects. We investigated key points in G-protein coupled receptor (GPCR) signaling, downstream of T2Rs. In mouse intestinal STC-1 cells, bitter compounds modulated the GPCR signaling cascade, downstream hormonal signals of carbohydrate consumption, and cell membrane expression of various bitter and glucose receptors. In C57Bl/6J mice, concurrent administration of bitter compounds and glucose load resulted in decreased absorption of glucose in the GI tract, with a subsequent rise in GLP-1 and CCK levels. Together, these data suggest bitter receptor stimulation results in transient delayed and increased insulinotropic signaling for rapid utilization of

blood glucose. These findings could emphasize the benefits of bitter phytochemicals in the diet and explain how these compounds improve postprandial glucose levels.

P-31

Underground Signaling Networks. PHILIP N. BENFEY. Biology Department and HHMI, Duke University, Durham, NC and Hi Fidelity Genetics, Durham, NC. Email: philip.benfey@duke.edu

To understand the progression from stem cells to differentiated tissues we are exploiting the simplifying aspects of root development. We have developed new experimental, analytical and imaging methods to identify networks functioning within different cell types and developmental stages of the root. We are particularly interested in a subnetwork that regulates a key asymmetric cell division of a stem cell and the regulatory networks that control differentiation of the stem cell's progeny. These networks are partially dependent on cell-to-cell signaling through movement of transcription factors. To quantify dynamic aspects of these networks, we are employing light-sheet microscopy to image accumulation of their different components. To find additional signaling molecules we performed ribosome profiling and identified putative peptide ligands. We have also uncovered a clock-like process responsible for the positioning of lateral roots along the root primary axis. Two sets of genes were identified that oscillate in opposite phases at the root tip and are involved in the production of prebranch sites, locations of future lateral roots. A derivative of the carotenoid biosynthesis pathway appears to act as a new mobile signal regulating root architecture.

P-32

How to Build a Business While You are Deciding on the Perfect Product or Technology to Commercialize? DAVID REED. Mimetics, LLC, 6 Davis Drive, Durham, NC 27709. Email: david.reed@mimeticsbiosci.com

Looking at biotech startups who have already succeeded is likely to give entrepreneurs beginning a business with a false sense of how businesses evolve. Almost by definition, success comes to a start-up when it finds a good product/market fit, but a close look will show that few if any businesses begin with that combination. So how do you build a business while you are seeking or testing out an idea or ideas for the product you are going to commercialize? The avenues are there, but the strategy has to be worked out carefully and it impacts everything from the size and composition of the team to the approach to raising capital.

P-33

Start Up for Commercialization, Idea Development, and IP Capture: How to Obtain Venture Capital. JEFFREY L. ROSICHAN. AgTech Accelerator Corp., 6 Davis Drive, Research Triangle Park, NC 27709. Email: jrosichan@agtechaccelerator.com

Creating a start up takes technology, talent, capital and facilities. Raising capital can be one of the biggest challenges faced by new entrepreneurs. Early funding can be obtained from a variety of public and private sources. Recently, there has been an explosion of incubators and accelerators to assist entrepreneurs to get their ideas more "venture ready". Having knowledge about what venture investors are looking for can help to better position your company for successful funding. Several strategies to help founders address these challenges will be discussed.

P-34

Intellectual Property Protection for Early Stage Companies. A. M. BONNEN. Myers Bigel, PA. 4140 Parklake Ave. Suite 600, Raleigh, NC, 20612. Email: abonnen@myersbigel.com

Start-up companies must consider many issues when they are establishing themselves; what products or services to be commercialized and market analysis for those products and services are, of course, paramount. In addition, obtaining funding for the company and establishing an IP portfolio are critical. IP protection and funding are highly interrelated as an early, well-developed strategy to protect the company's IP not only impacts competition but can be enticing to investors. On the other hand, failure to protect the IP that your company is built around can make the company very unattractive for investment. While IP is important for all companies, it is this interrelatedness with funding that makes a coherent and well thought out IP strategy especially vital to start-up companies. In order to develop an IP strategy one needs to have an understanding of intellectual property law. IP law encompasses the very different areas of trademarks, trade secrets, copyrights and patents. This presentation will focus on patent law. A brief overview of the basic requirements for patentability of an invention will be provided, including the threshold question of what constitutes patentable subject matter, an area of patent law that is currently in flux. I will discuss the types of patent applications available in the U.S. and worldwide, the situations where one type may be more advantageous than another, and the typical costs. In addition, mistakes that are often made, which can affect the quality or even the availability of protecting your IP, will be discussed so that they might be avoided.

P-35

Seizing Opportunities in a Complex Genome for Targeted Mutagenesis or Allele Replacement in Sugarcane. FREDY ALTPETER, Baskaran Kannan, Je Hyeong Jung, Tufan Mehmet Oz, Ratna Karan, and Aldo Merotto. Agronomy Department, Plant Molecular and Cellular Biology Program, University of Florida - IFAS, Gainesville, FL. Email: altpeter@ufl.edu

Programmable endonucleases like RNA-guided nucleases (e.g.CRISPR/Cas9) or transcription activator-like effector nucleases (TALENs) enable precise genome modifications. Targeted mutagenesis for "loss of function" is typically more efficient than gene replacement for "gain of function" since it does not require template mediated homology directed repair (HDR) and instead relies on the more efficient but error prone non-homologous end joining (NHEJ) DNA-repair pathway. However, sugarcane is a highly polyploid species (x=10–13) which may require knock-out of a very large number of alleles/copies for construction of "loss of function" mutants. Suppression of lignin biosynthesis can improve bioethanol production from lignocellulosic biomass. Recently we described TALEN induced mutagenesis to suppress one of the lignin biosynthetic genes, caffeic acid O-methyltransferase (*COMT*), resulting in low lignin and brown-midrib sugarcane phenotypes (Jung and Altpeter 2016, Plant Mol. Biol. 92: 131–142). Here we will describe Sanger sequencing of long *COMT*amplicons from brown-midrib sugarcane, which allowed us to precisely determine the number of copies/alleles which were co-mutated. Data describing the cell wall composition of *COMT* mutants and their agronomic and conversion performance will be presented. A precision gene-editing approach for allele re-placement conferring "gain of function" will also be discussed, involving a DNA repair template facilitating homology-directed repair (HDR) and CRISPR/Cas9 as programmable endonuclease.

P-36

CRISPR/Cas9-based Gene Editing in Rice and Maize. BING YANG. Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA 50011. Email: byang@iastate.edu

CRISPR (clustered regularly interspaced short palindromic repeats)-derived genome enabling systems, comprising Cas (e.g., Cas9, Cpf1) endonucleases and guide RNA (gRNAs) molecules have emerged as potent biotechnological tools for both basic and applied research. The most promising utilization of CRISPR technologies is for targeted genome editing, precise genetic alterations within any genome of interest, as demonstrated in a plethora of organisms including several crop plants. My presentation describes development and application of CRISPR technologies to generate heritable genome modifications in maize and rice. The CRISPR systems have been successfully established and applied to rice and maize for targeted mutagenesis of many genes. Transgenic lines of T0 generation carrying site-specific mutations were produced at frequency as high as 100% in rice and 80% in maize. Our results demonstrate that CRISPR systems are effective toolboxes for genome editing in rice and maize, empowering the discovery of gene function and the trait improvement.

P-37

Optimizing Gene Targeting in Plants. DAN VOYTAS. Department of Genetics, Cell Biology & Development and Center for Genome Engineering, University of Minnesota, Minneapolis, MN 55455. Email: Voytas@umn.edu

The ability to precisely modify plant genomes through homologous recombination (HR) promises to advance both basic and applied plant biology. However, even with the use of sequence-specific nucleases, which stimulate HR by creating targeted DNA double-strand breaks, there are only a handful of studies that report precise editing of endogenous plant genes. Our group has been focusing on two efforts to more effectively modify plant genomes through HR. In one, we are developing new vectors to deliver sequence-specific nucleases and DNA repair templates to plant cells. Specifically, we have been using geminivirus replicons, which function in both monocots and dicots, to amplify nuclease-encoding cassettes and DNA repair templates. In a second effort, we are attempting to achieve HR by either genetically manipulating DNA repair pathways or delivering nucleases and repair templates to cells proficient in HR. Progress on our efforts to optimize gene targeting strategies will be reported.

P-38

Development of Precision Genome Engineering Technologies in Maize. SHUJIE DONG and Qiudeng Que. Syngenta Crop Protection, LLC., 9 Davis Drive, Research Triangle Park, NC 27709–22571. Email: qiudeng.que@syngenta.com

Maize is an important food and feed crop in major agricultural production regions. Maize has benefitted greatly from genetic improvement through application of modern breeding technologies including transgenic technolgoies. Several important traits such as insect resistance and herbicide tolerance have

been engineered through the use of transgenes. In recent years breakthroughs in programmable nuclease technologies have made more direct and efficient to understand the molecular mechanism of various traits. The new genome engineering tools also made the genetic improvement of crops more precise. We have used maize crop as a model system to develop genome engineering tools for trait gene discovery, validation and variety improvement. This poster introduces our efforts in evaluating the performance of different nuclease options, development of high throughput editing event analysis capability, and enhancement of precise allele replacement and targeted insertion.